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Modulation of neuronal excitability in ex-vivo and in-vitro systems

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The main underlying mechanisms of chronic pain are still unclear. In this thesis, two new approaches targeting the excitability of peripheral nociceptive neurons were employed to examine processes leading to ongoing nociceptive discharge. Thereby we aimed to contribute to the understanding of peripherally driven spontaneous pain that leads to suffering in many patients with chronic pain. We used a new model of low extracellular potassium application that induces ongoing nociceptor activity at the sensory endings of skin nociceptors and a slow depolarizing ramp stimulus that preferentially activates C-fibres and is more closely related to the physiologic induction of action potentials in primary afferent neurons.
Low potassium solution unexpectedly led to a rapid and transient depolarisation that is probably mediated by a loss of ion selectivity in 2 pore domain potassium channels. However, the dominant effect of low potassium was a massive increase of intracellular sodium mediated primarily by an influx of sodium ions via NaV1.9 channels. Moreover, primary afferents lacking $Na_V1.9$ such as cold sensitive A-fibres of the cornea did not increase their discharge upon stimulation with low potassium.
Slow depolarizing ramp stimuli (4 Hz sinusoidal) were found to be more effective at activating C-fibres during cooling whereas traditional rectangular stimulation became less effective. This differential effect could be explained by cold-induced closure of two pore domain potassium channels leading to increased membrane resistance. The increased membrane resistance increases the membrane time constant whereby the same transmembrane current will more effectively change membrane potential. This mechanism has major clinical importance for cold-induced pain.
The voltage-sensitive sodium channel $Na_V1.7$ would have the ideal characteristics to mediate the activation upon slow depolarizing ramps as it is known to amplify such stimuli via a “ramp current”. Activation of C-fibres by sinusoidal stimuli was not blocked by TTX suggesting that $Na_V1.7$ is not the only Na_V that can respond to slow depolarizing changes in membrane potential.
Innovative excitability tests were used to investigate peripheral nerve fibres of rats that had received cisplatin to induce an acute nephropathy and potentially a peripheral neuropathy. In treated animals, unmyelinated fibres were found to be more sensitive to slow depolarizing stimuli suggesting hyper-excitability. However, the number of animals was low and it remains unclear how robust this effect is.
In summary, the model of potassium free extracellular solution was used to identify $Na_V1.9$ and 2 pore domain potassium channels as major determinants of small fibre excitability that have already been linked to clinical chronic pain states. Moreover, the cold-induced hyper-excitability of unmyelinated fibres in response to slow depolarizing ramp stimuli found in our study has important mechanistic implications for the generation of cold-induced pain and hypersensitivity in patients with neuropathic pain. It will be of major interest to use the newly developed tests for axonal excitability in pathophysiologic conditions and ultimately in human tissue in order to specify promising molecular targets that can lead to innovative analgesic treatment options in the future.